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# THE REGULATION OF ASEXUAL REPRODUCTION AND INDETERMINATE BODY SIZE IN THE SEA ANEMONE *ANTHOPLEURA ELEGANTISSIMA* (BRANDT)

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Certain sea anemones and related anthozoans reproduce asexually to form aggregated or dispersed clones. Such proliferation takes a variety of paths including binary longitudinal fission, transverse fission, pedal laceration, and regeneration from tentacles (reviewed by Sephenson, 1929; Chia, 1976). Little is known about the control of division rates or regulation of the size at which division occurs. The anemone *Haliplanella luciae* divides primarily by longitudinal fission, increasing the rate of fission as feeding rate increases (Minasian, 1976) and dividing at a larger size at higher temperatures and under conditions simulating periodic intertidal exposure (Johnson and Shick, 1977). Smith and Lenhoff (1976) have shown that a small acontiarian anemone will increase its rate of pedal laceration when starved. There appears to be no one common regulator of division across species and no information on the control of asexual proliferation in the field.

The sea anemone *Anthopleura elegantissima* (Brandt), common in the Pacific intertidal zone of North America, divides by binary longitudinal fission to form clonal aggregations (Hand, 1955; Francis, 1973a; Sebens, 1977), which recognize their own members and defend their space against non-clonemates during agonistic encounters (Francis, 1973b, 1976). Division can occur simultaneously among many individuals in a clone (Hand, 1955; Sebens, 1977). Pearse (1974), during a study of photosensitive behavior, noted that individuals divided in both dark and light treatments and that, while a few more divided in the light, the numbers were not significantly different.

Clonal aggregations can be composed of individuals of very different sizes, and mean size of clonal individuals can vary widely across habitats (*e.g.*, 0.8–4.2 cm, Sebens, 1977). Mean individual size increases with decreasing intertidal height, indicating that individuals are probably dividing at a smaller size in the high intertidal. Observation of mapped clones and examination of recent division scars have shown that most division takes place from August to March and that it is primarily the larger individuals in a particular clone that undergo fission (Sebens, 1977). Individuals do not appear to divide until they are at least 1.0–1.2 cm basal diameter, approximately 2 years or more after setttlement. Anemones that have divided are shrinking or plateauing in size during August to March, with the greatest growth occurring from April to July. A theoretical model for optimal size at division and optimal size of colony units in anthozoans has been presented and discussed as it applies to *A. elegantissima* (Sebens, 1979).

Sexual reproduction occurs annually in *A. elegantissima* (Ford, 1964; Sebens, 1977; Jennison, 1977, 1979) and most individuals above 1.2-cm basal diameter are fertile at the peak of the season (July, August; Sebens, 1977). Clone members are all of one sex (Ford, 1964; Jennison, 1977; Sebens, 1977) although a few hermaphrodites have been found at one site (Jennison, 1977). Gonad production begins around January and spawning occurs in August to September (Ford,

1964; Sebens, 1977; Jennison, 1977). Given the high percent fertility (94–100%, Sebens, 1977) of individuals greater than 1.2 cm basal diameter, it is almost certain that some of the same individuals that reproduce sexually undergo fission the following autumn or winter. Very few individuals (< 1%) undergo more than a single fission per season (Sebens, 1977).

The present study was designed to investigate the control of body size at division and the timing of fission in *A. elegantissima*. Specifically, the effects of light, temperature, and feeding regime on weight change and division rate were examined in laboratory aquaria using an experimental group of 1000 anemones. Light regime (irradiance) affects the energetics of *A. elegantissima* through the zooxanthellae which supply part of the anemone's nutrition (Muscatine, 1961). Temperature varies as a factor of season, intertidal height, length of exposure and depth of the tide pools (Dayton, 1971) and feeding rate changes with season, as reflected in the growth and shrinkage rate of anemones in the field (Sebens, 1977).

## Materials and Methods

In March, 1976, 1500 specimens of *A. elegantissima* of a full size range were collected at Cattle Point, San Juan Island, Washington (48°27'N, 22°57'W). The anemones were transported to sea-water aquaria at the University of Washington, Seattle, where they were examined for division scars, measured, and kept under observation for 2 weeks. Twenty plastic basins (10 l each) were distributed among constant temperature rooms or provided with aquarium heaters to adjust temperature. All were aerated and half were covered with opaque black plastic sheeting on the top and sides. The other half were covered by clear plastic sheeting, placed under two fluorescent bulbs (40 watt) at 15 cm above the tanks (70–100 microeinsteins per $m^2$/sec). Five temperatures were chosen (5, 10, 15, 20, 25° C) spanning the range that the anemones normally encounter in intertidal pools (Dayton, 1971). With light and dark treatments at each temperature, and fed and starved groups for each of those 10 treatments, 20 separate treatments (aquaria) resulted.

Fifty anemones (undamaged and covering the size range) were placed in each basin filled with filtered (particles $\geq 20$ $\mu$m removed) sea water. Feeding was every other day for half of the treatments (500 anemones). Pieces of mussel (*Mytilus californianus*, tissue fragments 0.2–0.5 g wet weight) from frozen stocks were fed with forceps to each anemone. The following day all pieces of egested or rejected food were removed by suction with a large plastic syringe. Water was changed every fourth day (after every second feeding) and most of the developing algal layer on the basin surfaces was removed at that time with a paper towel.

Anemones were observed daily, noting any that began a division or appeared to do so. Early potential dividers showed elongation of the pedal disc (most did not go on to divide). Intermediate potential dividers elongated the oral aperture as well (most divided). Actual dividers had begun separation of the halves. Post dividers had completed the division, showing new division scars. Sizes of anemones at any stage of potential division were measured (length and width of pedal disc) and their positions were drawn on data sheets with oriented maps of each tank. All anemone sizes were measured at 0, 34, and 50 days of the experiment.

A plastic-screened enclosure holding ten anemones (approx. 1.5-cm diameter each) was placed in the center of each basin. This constituted the weight monitored group. Anemones were identified with small neutral red dye spots (Sebens,

1976) and were easily removed from the smooth plastic surface. They were weighed in sea water (on a torsion balance) by the reduced weight method (Zeuthen, 1948; Muscatine, 1961) followed by a rapid weighing in distilled water to calculate any change in anemone tissue density during the course of the experiment. Anemones were hung on fine stainless steel wire suspended from a torsion balance in a beaker of seawater (from a filtered stock kept in the dark for the duration of the experiment). Treatments ran for a total of 60 days (April–May, 1976).

## Results

### *Weight change*

Weight changes of specimens of *Anthopleura elegantissima* over a 30-day period, expressed as percent change, were submitted to an analysis of variance (single classification, Model I) to ascertain the effect of all treatment variables. Means, 95% confidence limits for the mean, and the analyses of variance were run using both the original data and arcsine transformations of the data suggested for percentages and ratios (Sokal and Rohlf, 1969). The transformation used was as follows: $\theta = \arcsin\ ((P + 100)/200)^{0.5}$, where p is the original percent weight change.

Table I

*Descriptive statistics for 30-day percent weight change of* Anthopleura elegantissima. *$X\%$, $s$, $L_1$, $L_2$ are mean, standard deviation, and upper and lower 95% confidence limits of the mean for percent weight change data. The equivalent statistics were calculated for arcsine transformed data, where $0° \leqslant \theta \leqslant 90°$. $X_{arc}$, $L_1$, $L_2$ are mean, and upper and lower 95% confidence limits for percent weight change, calculated by back-transforming the mean and limits determined for $\theta$. Treatments are noted by temperature (5°, 10°, 15°, 20°, and 25° C), feeding regime (fed (F), starved (S)), and illumination (light (L), dark (D)).*

| Treatment | Percent change | | | | Percent change (arcsine) | | |
|---|---|---|---|---|---|---|---|
| | $\bar{X}\%$ | s % | $L_1$ | $L_2$ | $\bar{X}_{arc}$ | $L_1$ | $L_2$ |
| 5° SD | −23.4 | 6.2 | (−29.1, | −17.7) | −23.5 | (−29.1, | −17.8) |
| 5° FD | − 1.9 | 16.9 | (−17.5, | +13.7) | − 1.9 | (−17.5, | +13.7) |
| 5° SL | −11.4 | 10.0 | (−20.6, | − 2.2) | −11.4 | (−20.6, | − 2.2) |
| 5° FL | +24.5 | 34.0 | (− 6.9, | +58.5) | +32.1 | (−18.2, | +74.3) |
| 10° SD | −31.4 | 6.0 | (−37.0, | −25.8) | −31.5 | (−37.0, | −25.8) |
| 10° FD | +15.8 | 21.0 | (− 3.5, | +35.2) | +16.3 | (− 4.0, | +35.8) |
| 10° SL | −12.9 | 9.4 | (−21.5, | − 4.2) | −12.9 | (−21.6, | − 4.2) |
| 10° FL | +48.8 | 37.3 | (+14.4, | +83.2) | +51.5 | (+13.0, | +81.5) |
| 15° SD | −28.5 | 15.9 | (−43.1, | −13.9) | −28.9 | (−43.6, | −13.5) |
| 15° FD | + 0.9 | 21.4 | (−18.9, | +20.7) | + 0.9 | (−19.0, | +20.9) |
| 15° SL | −23.9 | 8.2 | (−31.5, | −16.4) | −24.0 | (−31.5, | −16.4) |
| 15° FL | +15.7 | 8.1 | (+ 8.3, | +23.2) | +15.8 | (+ 8.3, | +23.2) |
| 20° SD | −39.7 | 8.9 | (−47.9, | −31.5) | −39.9 | (−47.9, | −31.5) |
| 20° FD | −27.5 | 12.1 | (−38.6, | −16.3) | −27.7 | (−38.8, | −16.2) |
| 20° SL | −42.5 | 10.0 | (−51.7, | −33.2) | −42.7 | (−51.7, | −33.2) |
| 20° FL | −37.2 | 13.0 | (−49.2, | −25.2) | −37.6 | (−49.5, | −25.0) |
| 25° SD | −44.0 | 22.3 | (−64.6, | −23.4) | −45.0 | (−64.0, | −23.7) |
| 25° FD | −41.6 | 10.2 | (−51.1, | −32.2) | −41.9 | (−51.1, | −32.2) |
| 25° SL | −41.9 | 8.6 | (−49.8, | −34.0) | −42.1 | (−50.0, | −33.9) |
| 25° FL | −46.5 | 5.6 | (−51.7, | −41.3) | −46.6 | (−51.8, | −41.3) |

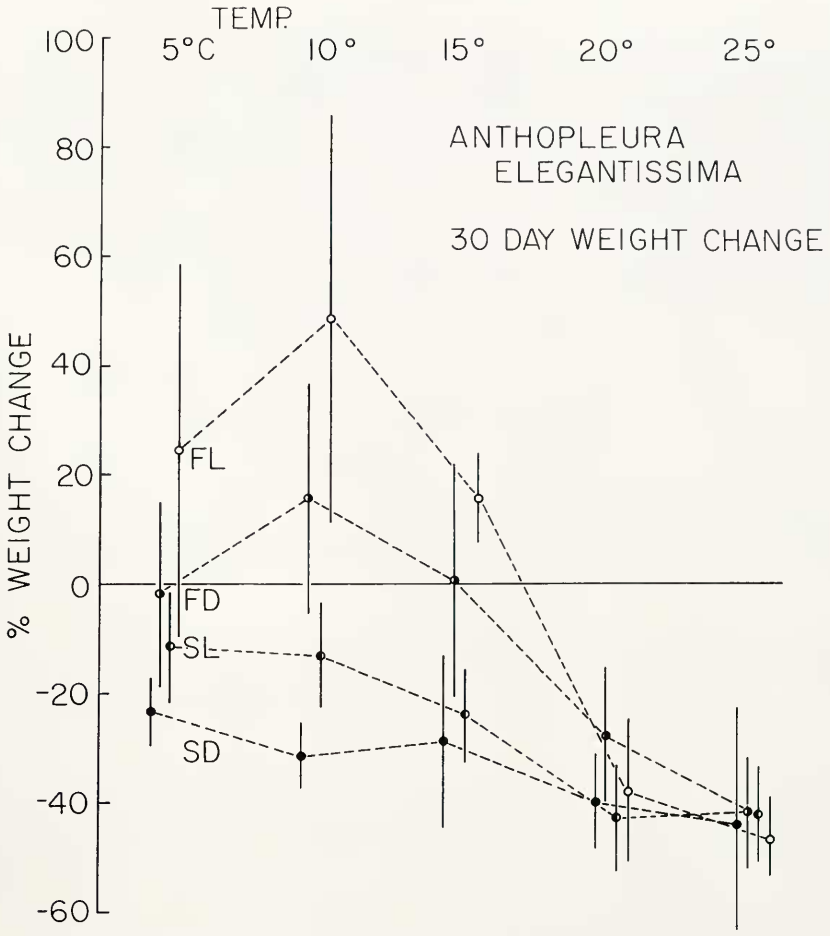


FIGURE 1. Weight change of groups of seven specimens of *A. elegantissima* in each experimental treatment in the laboratory (± standard deviation) expressed as percent original weight.

Table I compares the mean, standard deviation, and 95% confidence limits for the mean as calculated for the original percent data and from the transformation, $\theta$. Only the values for the 5° C group fed in the light and the 10° C fed in the light gave somewhat different estimates by the two methods.

Assumptions made in performing an analysis of variance are met to some extent by the data. Individuals used in the weight change experiment were a blind choice from all available individuals. The experimental design was such that aquaria were identical in construction and their positions were independent with respect to external influences. The assumption of homogeneity of variances (homoscedasticity) (Sokal and Rohlf, 1969) is met in some of the comparisons but not in others (F-max test). Analysis of variance was carried out on all the data, and on subgroups defined by feeding regime, illumination, temperature and finally as 1 to 1 comparisons of groups within each temperature. Calculations were done on an IBM 360 computer using the SPSS subprogram ANOVA (Nie, Hull, Jenkins, Steinbrenner and Bent, 1975). Two-way analysis of variance (Model I)

TABLE II

*Two-way analysis of variance on 30-day percent weight change of* Anthopleura elegantissima. *"Data group" denotes the portion of the total treatments used in the analysis. "Factors" are the subgroups to be compared in Model I ANOVA. N is the number of data points per subgroup and d.f. are degrees of freedom. Sums of squares are divided into the interaction sum of squares ($SS_2$) and total ($SS_t$). Mean squares are given as interaction mean square ($MS_2$) and total ($MS_t$). $F_s$ is given for percent change data, with its significance level. $F_s$, determined by carrying out the same ANOVA on arcsine transformed percent change data, is also given with its significance level. * denotes significance at the $P < 0.05$ level, ** at the $P < 0.01$ level, *** at the $P < 0.001$ level. Subgroups are denoted by the following: feeding regime (Fd) including fed and starved groups, illumination (I) including light and dark groups, and temperature (T) including treatments at 5°, 10°, 15°, 20°, and 25° C.*

| Data group | Factors | N | d.f. | $SS_2$ | $SS_t$ | $MS_2$ | $MS_t$ | $F_s$ | $F_{s\theta}$ | sig. $F_s$ | sig. $F_{s\theta}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All | I, Fd | 140 | 1 | 0.2 | 127.8 | 0.2 | 0.9 | 0.3 | 0.5 | 0.604 | 0.494 |
| All | T, Fd | 140 | 4 | 13.2 | 127.8 | 3.3 | 0.9 | 10.3 | 7.5 | 0.000*** | 0.000*** |
| All | T, I | 140 | 4 | 5.0 | 127.8 | 1.2 | 0.9 | 2.3 | 2.2 | 0.060 | 0.070 |
| Fed | T, I | 70 | 4 | 4.8 | 88.9 | 1.2 | 1.3 | 2.9 | 2.4 | 0.029* | 0.064 |
| Starved | T, I | 70 | 4 | 1.0 | 17.1 | 0.2 | 0.2 | 1.9 | 1.6 | 0.129 | 0.176 |
| Light | T, Fd | 70 | 4 | 9.9 | 84.8 | 2.5 | 1.2 | 8.0 | 5.0 | 0.000*** | 0.002*** |
| Dark | T, Fd | 70 | 4 | 4.1 | 40.0 | 1.0 | 0.6 | 12.3 | 12.3 | 0.000*** | 0.000*** |
| 5° | I, Fd | 28 | 1 | 0.4 | 18.2 | 0.4 | 0.7 | 0.9 | 0.8 | 0.347 | 0.394 |
| 10° | I, Fd | 28 | 1 | 0.3 | 36.0 | 0.3 | 1.3 | 0.7 | 1.1 | 0.416 | 0.298 |
| 15° | I, Fd | 28 | 1 | 0.2 | 14.3 | 0.2 | 0.5 | 0.9 | 0.9 | 0.359 | 0.396 |
| 20° | I, Fd | 28 | 1 | 0.1 | 3.9 | 0.1 | 0.1 | 0.7 | 0.6 | 0.414 | 0.429 |
| 25° | I, Fd | 28 | 1 | 0.1 | 4.4 | 0.1 | 0.2 | 2.8 | 2.7 | 0.475 | 0.497 |

was also used to examine interaction effects between the three treatment variables. All analyses were carried out on the original percent change data (made positive by addition of a scalar, 100), and on the arcsine transformed data. Results of the two-way analysis are given in Table II with the F values and their significance levels supplied by the SPSS program. Complete results of the one-way analysis are available from the author upon request.

The results of the analyses of variance will be discussed as they relate to each treatment variable: Temperature significantly affected weight change when all data were subjected to the analysis ($P < 0.001$), within each of the feeding regimes ($P < 0.001$) and within each of the illumination regimes ($P < 0.001$). Illumination did not significantly affect weight change when all data were subjected to the analysis, or when only the fed or starved groups were used. It did affect weight change significantly only in the 5° C temperature group and in the 5° and 10° C starved groups when temperature groups were broken down for one-to-one comparisons. Feeding regime had a significant effect on weight change when all data were used in the analysis ($P < 0.001$), within the light ($P < 0.001$) and dark ($P < 0.001$) groups and in each of the temperature groups except those at 20° and 25° C. When temperature groups were broken down for one-to-one comparisons, feeding regime had a significant effect in all groups except those at 20° and 25° C.

Two-way analysis of variance indicated a significant interaction effect ($P < 0.001$) between temperature and feeding regime within all groups tested. A significant interaction also occurred between temperature and illumination in the fed group ($P < 0.05$). No significant interaction was indicated between feeding regime and illumination when all data were used, or in any of the temperature

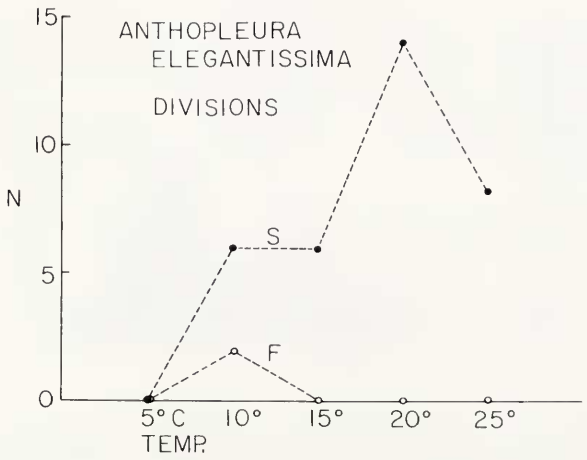


FIGURE 2. Numbers of divisions in each treatment during laboratory experiments with *A. elegantissima*. Starved (S) and fed (F) groups are compared at each experimental temperature. Number of divisions are those that occurred over the total 60-day period. Maximum number of divisions was 50 per treatment.

treatments. The last result is interesting since feeding and illumination both affect energy intake, and can be presumed additive, while temperature affects food assimilation, feeding behavior, and metabolic cost and would not be presumed to have an additive effect with food or illumination.

Differences between light and dark groups agree well with Muscatine's (1961) measurements of weight loss in starved specimens of *A. elegantissima* with and without zooxanthellae. At 20° and 25° C all groups lost weight at a comparable rate, independent of treatment. At the lowest temperature, 5° C, anemones grew or shrank less than at 10° and 15° C. At that low temperature, activity was reduced and food was often rejected or egested with little apparent change. If symbiotic algae (zooxanthellae) produce oxygen more rapidly at higher temperatures, but also metabolize more of their photosynthetic products, zooxanthellae would be a liability and anemones would lose weight more rapidly because of increased metabolic rate. Metabolic rate increases with oxygen tension and temperature for anemones (Sassaman and Mangum, 1972; Sassaman 1973, 1974; Brafield and Chapman, 1967; Beatties, 1971). At 25° C most of the anemones ejected their algal symbionts after two weeks. Pearse (1974) was able to produce algae-free specimens for experimental use by keeping them at similar raised temperatures. Goreau (1964) and Reimer (1971) have noted that various anthozoans expel zooxanthellae during extreme stress. This result suggests that the intracellular symbionts are rejected when their presence begins to subtract from, rather than add to, the host's energetic budget.

Density change of anemone tissue was monitored by weight difference in two fluids (sea water and distilled water). The reduced weight technique is accurate for measuring weight change only if tissue density does not change substantially during the experiment (*e.g.*, if only lipid were lost, it would make tissue more dense). Weight (g) in sea water ($W_s$) is given by: $W_s = (D_t V_a) - (D_s V_a)$ where $D_t$ is density (g/cm$^3$) of tissue, $D_s$ is the density of distilled water and $V_a$ (cm$^3$) is volume of anemone tissue, equal to water displaced when the anemone is immersed for weighing. Weight in distilled water ($W_f$) is: $W_f = (D_t V_a) -$

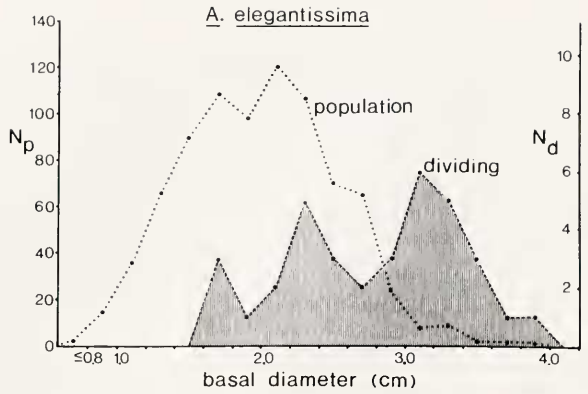


FIGURE 3. Sizes of specimens of *A. elegantissima* dividing ($N_d$ scale) during laboratory experiments. Size-frequency distribution of the entire experimental population ($N_p$ scale). Mean diameter of the experimental population was 2.0 ± 0.5 cm s.d. ($N = 730$). Individuals that divided had a mean diameter of 2.9 ± 0.5 cm s.d. ($N = 34$).

($D_f V_a$) where $D_f$ is the density of distilled water. The ratio of $W_s$ to $W_f$ is: $W_s/W_f = (D_t - D_s)/(D_t - D_f)$ where the $V_a$ term is removed. The ratio will obviously change if $D_t$ changes during weight change of the anemone. The value of $W_s/W_f$ at the beginning of the experiment was 0.54 ± 0.11 s.d. for the groups at 5° C fed and starved in the dark (N = 18) and 0.47 ± 0.07 s.d. for the same groups after 34 days. No significant change had occurred in the ratio (Student's t-test, t = 2.0, not significant). $W_s/W_f$ does not differ from a normal distribution in the groups before or after weight loss (Chi-square test, d.f. = 19).

*Frequency of asexual division*

Division frequencies were not significantly different in light and dark treatments (19 light, 17 dark) but were very different in the fed and starved groups (34 starved, 2 fed) (Fig. 2). A Chi-square test of number of individuals dividing per group of 50 showed significance for all fed vs. all starved groups ($\chi^2 = 28.44$, d.f. = 1, $P < 0.0001$), for different temperature treatments within the starved group ($\chi^2 = 14.82$, d.f. = 4, $P < 0.01$) but not for different temperatures within the fed group. Within the starved groups, division frequency increased with temperature to 25° C, where divisions were fewer. At 25° C many of the anemones died and others showed signs of physiological stress (everted actinopharynx, reduced sensitivity to prodding) and the entire division process may have been impaired. At 20° and 25° C, even though fed groups were actually losing weight, they were not dividing. Possibly the presence of food inhibited division while increased metabolic rate stimulated division only when individuals were not feeding.

Only the larger anemones divided during the experiment (Fig. 3). The experimental population had a mean initial individual diameter of 2.1 ± 0.6 cm s.d. while the anemones that divided had a predivision diameter of 2.9 ± 0.5 cm s.d. Anemones that did not divide during the course of the experiment began at a mean size of 2.0 ± 0.5 cm s.d. Dividing anemones were significantly larger than those that did not divide (Student's t-test, $P < 0.001$). This agrees well with the sizes of anemones that divided (1.9 ± 0.4 cm s.d.) and did not divide (1.5 ± 0.4 cm s.d.) in the field (Sebens, 1977), determined by comparing those with and

without division scars (signficantly different by Student's t-test, $P < 0.001$). The sizes of anemones that divided during the experiment were significantly smaller at 20° C (2.0 ± 0.5 cm s.d.) and 25° C (2.0 ± 0.6 cm s.d.) than at 10° C (2.5 ± 0.6 cm s.d.) and 15° C (2.6 ± 0.5 cm s.d.). (Student's t-test, $P < 0.05$). Sizes of anemones dividing at 20° and 25° C were not significantly different from each other nor were those at 10° and 15° C.

Increased metabolic cost due to raised temperature may have stimulated division rate in the experimental aquaria. In the field, largest individual size within a clone correlates with observed temperature. Figure 4 compares maximum temperatures reached by anemones in 15 tide pools exposed at low tide on a single day with the mean size of the ten largest individuals in each pool. All pools (Iceberg Point, Lopez Island) were on a flat bench within (0.09 m) intertidal height, and so experienced the same immersion time for feeding. The pools thus differ primarily in temperature while exposed, influencing metabolic cost, which increases with temperature. In fact, pools which reach lower temperatures due to shading or depth have the larger anemones. That is, division probably occurred at a smaller size in pools that reach higher temperatures. High-intertidal anemones experience generally greater temperatures when exposed than do those in the low intertidal. Consequently, the former are smaller at both sites in the San Juan Islands (Sebens, 1977).

## Discussion

Asexual division is inhibited when *A. elegantissima* is fed continuously. Such proliferation occurs more rapidly and in a larger fraction of the population as ambient water temperature increases. Starvation, defined as weight loss, is not in itself sufficient stimulus to initiate the division process. If shrinking individuals are given food, even though it may not be efficiently assimilated (as at 20° and 25° C) (Fig. 1), division still does not occur. Light or darkness has no significant effect on the division process although it has a marked effect on weight

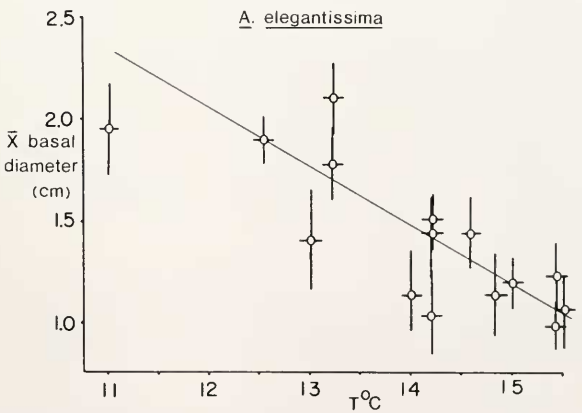


Figure 4. Mean basal diameter (± 95% confidence interval) of *A. elegantissima* in high tidepools (+ 1.8 m to + 1.9 m) at Iceberg Point, Lopez Island, plotted against maximum temperature (± range) reached by the pool at a given low tide. Mean basal diameters are the mean of the ten largest anemones in each pool. The regression line is: basal diameter (mm) equals − 2.3T(° C) + 46.4 with r = 0.77.

change. Finally, it appears that only the larger members of the population undergo fission, as in the field (Sebens, 1977).

Weight change during the experiment was significantly affected by temperature, feeding regime, and illumination (Fig. 1). Muscatine (1961) found that *A. elegantissima* lost weight less rapidly in the light when it had zooxanthellae, thus implicating the zooxanthellae as a positive factor in the anemone's energetic regime. This result is supported here in a slightly different manner. All anemones had zooxanthellae at the beginning although many of the anemones at 25° C egested them during the course of the experiment. Weight gain in fed anemones was greater and weight loss during starvation less when given a high level of irradiance, again pointing to a significant nutritional role for the zooxanthellae.

The effect of illumination and feeding regime changed at 20° and 25° C, at which point all treatment groups lost weight rapidly. Zooxanthellae, mostly retained at 20° C, are no longer an advantage and may in fact become an energetic burden. At 5° C, prey digestion or assimilation efficiency must be reduced since weight gain was much less than at 10° C. However, metabolic rate must also be lower and thus weight loss in starved groups was less at 5° C than at any of the other temperatures. Temperatures do get this low during winter exposures in the intertidal (Dayton, 1971) and the anemones may be energetically shut down for the season. Size changes in the field support this contention (Sebens, 1977). Continuous exposure to 5°, 20°, or 25° C is, however, outside of the range experienced by *A. elegantissima*.

The most interesting weight changes occurred at 10° C, a common ambient temperature for the anemones during much of their growth and reproductive season (Dayton, 1971; Sebens, 1977) on the Washington coast. The separation of the treatment effects was the greatest and most significant and the zooxanthellae thus had their greatest effect on anemone energetics in both fed and starved individuals. Feeding produced the greatest weight gain at this temperature even though starvation caused shrinkage at a rate slightly greater than at 5° C. The light level used, well below that which can be encountered in the intertidal (yet constant over 24 hr), produced a weight gain in fed animals greater than twice that in the dark and a weight loss during starvation less than half that in the dark.

The results of the laboratory experiments agree well with the field observations on individual size within clones and timing of division. Division occurs during the time of year when temperatures are relatively low (6°–10° C), prey is least available, and there is time for the necessary internal reorganization that must occur before the next feeding period. During the months of August through December, individuals are generally shrinking, as measured by basal diameter (Sebens, 1977). Both mapped individuals in monitored clones and quadrat samples of the population at large showed this trend (six sites).

Starvation during the autumn and winter months could thus be a cue to initiate the division process, although prolonged lack of prey during any time of the year may do so in some members of the population which have wandered into particularly poor locations. The field studies showed a few dividing individuals even in the summer months. Individuals used for this set of experiments were collected during the winter when much of the population does undergo fission and when the sexual reproduction cycle is just beginning; hence gonads were not yet a confounding factor. It is not known whether fully developed gonads can act as a deterrent to division.

By contrast, other anemone species (*e.g., Haliplanella luciae*) can undergo fission repeatedly and are probably not tied to an annual cycle of the same type as is *A. elegantissima* (Minasian, 1976; Johnson and Shick, 1977). An unidentified acontiarian anemone (Smith and Lenhoff, 1976) and *Metridium senile* (Davis, 1920, and personal observation) form pedal lacerates repeatedly and show no apparent seasonality. *Anthopleura elegantissima* shares a similar morphological form of longitudinal fission with *Haliplanella luciae* yet the two respond very differently to ambient conditions. *H. luciae* divides more frequently when fed (Minasian, 1976) and divides at a larger size at higher temperatures (Johnson and Shick, 1977), exactly the reverse of *A. elegantissima*. The control of division is thus highly species-specific and very likely adapted to the ecological conditions over the range of habitats occupied by a particular species.

The present set of experiments gives some indication as to how control of size may come about. *A. elegantissima* is usually limited to a single division per individual per year. Mean size of individuals within clones thus will change with habitat if some habitat-dependent threshold size must be attained before division. In the laboratory experiments, more individuals per treatment divided as temperature increased from 5°–20° C. Individuals dividing at the lower temperatures were generally larger than those dividing at the higher temperatures. If the decision to divide is based on energetics (*e.g.,* they will divide at a smaller size if losing weight more rapidly), then a single annual division could produce the observed pattern of variable size. This situation has been used as an example of size regulation in a theoretical model of indeterminate growth (Sebens, 1979).

Some closely related anemones never divide asexually (*e.g., Anthopleura xanthogrammica* (Brandt), Sebens, 1977) and a variant of *A. elegantissima* in California which grows to a large size (at least 15-cm basal diameter) appears not to divide (Sebens, 1977; Francis, 1979). According to the results of these experiments, the large variant, if indeed the same species, could be produced by continuous feeding, removing the starvation cue for division. Whether or not this is actually the case, it is clear that there are situations where division is either impossible or somehow maladaptive. Elsewhere Sebens (1979) has shown that a large solitary individual can be energetically favored when the prey resource consists of large, relatively rare, prey items. Division would produce smaller individuals with greater total feeding surface but unable to use the large prey items. Both *A. xanthogrammica* and the large variant of *A. elegantissima* occur lower in the intertidal than the clonal *A. elegantissima* and both feed on large mussels (*Mytilus californianus*) while smaller *A. elegantissima* feed on a variety of intertidal invertebrates and zooplankton of relatively small size (Sebens, 1977). Inhibition of division in the case of large prey specialists could be facultative (a single species could be clonal or solitary depending on prey and habitat) or obligate, losing the ability to reproduce asexually over evolutionary time (*e.g., A. xanthogrammica*). A facultative case may occur in *A. elegantissima* and in a co-familial anemone in the Chilean intertidal, *Phymactis clematis* (Drayton) which produces both small clonal and large solitary individuals (Stotz, 1977; Sebens and Paine 1978). The ability to form clonal aggregations or dispersed clones may be advantageous in space occupation and defense (Francis, 1973a).

Asexual reproduction by fission shares more aspects with colony formation than with reproduction by small propagules. Williams (1975) describes this type of reproduction in his "strawberry-coral model" as a means of occupying nearby habitat space in an area already proven suitable for the "parental" genotype. This

appears to be a reasonable model for the clonal aggregations of sea anemones which colonize an area as a sexual propagule (planula larva) and then fill available space through asexual means. Such anemones usually produce gonads and sexual products annually, devoting as much of their relative biomass to this activity as do species which do not divide (Sebens, 1977). With external fertilization and planktonic larvae (Siebert, 1974), *A. elegantissima* is essentially unable to colonize even nearby areas with sexual offspring. All sexual propagules are used in long-range dispersal and all asexually produced individuals are retained in the same habitat if not always in the same aggregation.

Binary fission producing roughly equal daughter individuals is not the only option taken by anemones. Pedal laceration produces multitudes of small tissue fragments, each of which becomes a new anemone much smaller than the parent. Calow *et al.* (1979) have discussed the advantages of equal and unequal fission in freshwater flatworms where one part receives the pharynx and the other must regenerate everything anew, much the same as in pedal laceration where a lacerate must regenerate all internal structures and tentacle crown before it can begin feeding. This results in one relatively low risk and one or more high risk offspring, which may be better at times than two resulting units neither of which can feed for awhile. If vertical size were an advantage in either intraspecific or interspecific competition, this arrangement could be very important.

As is true for morphological attributes, the selective pressures on asexual proliferation can be numerous, even in a relatively simple process such as binary fission. Factors affecting timing and size at division have been considered but these are only a few of the potential variables involved. This form of asexual multiplication has not been represented as an alternative to sexual reproduction. On the contrary, it appears that the proper timing of division and regulation of indeterminate body size acts to maximize the energetic budget of *A. elegantissima* such that the output of gametes per clone at the end of the growing season is as great as possible.

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## Summary

The sea anemone, *Anthopleura elegantissima*, forms distinct clonal aggregations in the field by longitudinal fission (Hand, 1955, Ford, 1964, Francis, 1973a, b, 1976, Sebens, 1977). Field observations indicate that division takes place most

often in the fall and winter when individual anemones are decreasing in size (Sebens, 1977). Mean size of clonal individuals decreases with increasing intertidal height (Sebens, 1977) and with increasing tidepool temperature at a given height.

In the laboratory, asexual division is inhibited when *A. elegantissima* is fed continuously. Such division occurs more rapidly and in a larger fraction of the population as ambient water temperature increases. Starvation, defined as weight loss, is not in itself sufficient stimulus to initiate the division process. Shrinking individuals, given food, do not divide even though that food may not be efficiently assimilated (as at 20° and 25° C). Strong light or darkness has no significant effect on the division process although it has a marked effect on weight change. Finally, it appears that only the larger members of the population undergo fission, as in the field (Sebens, 1977).

Weight change in experimental groups is significantly affected by temperature, feeding regime, and illumination. Evidence that zooxanthellae are a significant positive factor in the anemone's energetic regime is supported. All anemones had zooxanthellae at the beginning although many anemones at 25° C egested them during the course of the experiment. Weight gain in fed anemones was greater, and weight loss during starvation less, when given illumination than in darkness.

The effect of illumination and feeding regime changed at 20° C and at 25° C, at which point all treatment groups lost weight rapidly. Analysis of variance indicates significant interaction between temperature and feeding regime, and between temperature and light (in the fed group) as factors affecting weight loss or gain. Light and feeding regime do not show significant interaction.

The results of laboratory experiments support the hypothesis that seasonal control of longitudinal fission is affected through the energetic regime, commencing during periods of starvation. More rapid weight loss and lesser feeding time in high intertidal pools may result in division at a smaller size and thus clones where mean individual size is smaller than in the low intertidal.

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